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SHORT REVIEW

Lowering the dexamethasone suppression test cut-off: is it time yet?

Vittoria Favero[1] · Cristina Eller-Vainicher[2] · Valentina Morelli[3] · Alfredo Scillitani[4] · Iacopo Chiodini[1,5]

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Abstract

Purpose Cushing's syndrome (CS) is a rare condition marked by overt hypercortisolism, while mild autonomous cortisol secretion (MACS) refers to asymptomatic or subclinical cortisol excess, often associated with adrenal incidentalomas (AI). MACS is significantly more prevalent than CS, particularly in older populations. The 1 mg overnight dexamethasone suppression test (F-1mgDST) is the main diagnostic tool, but the appropriateness of its current cut-off value of 1.8 µg/dL is under debate.

Methods This review briefly analyses existing literature to assess whether this threshold accurately identifies patients with cortisol-related comorbidities, especially in those with non-functioning AI (NFAI). We searched PubMed, Web of Science and Scopus between January 1990 and March 2025 using the following keywords: "adrenal incidentalomas, non-functioning adrenal incidentalomas, adrenal adenomas, non-functioning adrenal adenomas, subclinical hypercortisolism, mild hypercortisolism, less severe hypercortisolism, hidden hypercortisolism, mild autonomous cortisol secretion".

Results Evidence suggests that NFAI patients often present with metabolic, cardiovascular, bone and muscle alterations typical of cortisol excess, even with F-1mgDST values below 1.8 µg/dL. Several studies propose that a lower threshold (around 1.2 µg/dL) may better capture at-risk individuals, although this could reduce specificity and increase false positives.

Conclusions The review highlights the need for more sensitive diagnostic criteria, possibly including biomarkers and pharmacological challenge tests. It also introduces the concept of a cortisol "milieu" in eucortisolemic individuals, suggesting that even normal-range cortisol activity and/or altered cortisol circadian rhythm may contribute to chronic conditions. In light of current evidence, closer monitoring of AI patients with F-1mgDST above 1.2 µg/dL is recommended, pending validation from longitudinal and interventional studies.

Keywords Mild autonomous cortisol secretion · Adrenal incidentalomas · Cortisol after dexamethasone suppression test · Cortisol circadian rhythm · Cortisol sensitivity

Introduction

Cushing's syndrome (CS) is a rare disease characterized by the presence of a biochemical cortisol excess in the presence of specific signs and symptoms of hypercortisolism, such as easy bruising, facial plethora, proximal myopathy (or proximal muscle weakness), striae (especially if reddish purple and > 1 cm wide), and in children weight gain with decreasing growth velocity [1].

Since the late 90's a condition of biochemical hypercortisolism in the absence of the specific signs and symptoms of cortisol excess has been identified. This disorder has been defined with various terms (i.e. subclinical hypercortisolism, less severe hypercortisolism, mild cortisol excess, hidden hypercortisolism), the last used being "mild autonomous cortisol secretion" (MACS) [2].

✉ Iacopo Chiodini
iacopo.chiodini@unimi.it

1 Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan 20100, Italy

2 Unit of Endocrinology, Fondazione IRCCS Cà Granda-Ospedale Maggiore Policlinico Milan, Milan, Italy

3 Endocrinology Department & Lab of Endocrine and Metabolic Research, IRCCS, Istituto Auxologico Italiano, Milan, Italy

4 Unit of Endocrinology, Ospedale "Casa Sollievo della Sofferenza", San Giovanni Rotondo, Foggia, Italy

5 Unit of Endocrinology, ASST Grande Ospedale Metropolitano Niguarda, Piazza Ospedale Maggiore 3, Milan 20162, Italy

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The term MACS bases itself on the autonomy of the adrenal cortisol secretion since this condition is thought to be present mainly in patients with incidentally discovered adrenal masses (adrenal incidentalomas, AI). MACS is reported in up to 50% of AI, which, in turn, are found in up to 7% of subjects above 60 years of age. Given these frequencies, the prevalence of MACS in subjects above 60 years of age could be estimated to reach the 3.5% [3], importantly higher than that of CS (up to 79 cases/million) [4].

Prior to 2003, the biochemical diagnosis of CS was not clearly defined, being based on the presence of not suppressed cortisol levels after 1 mg overnight dexamethasone test (F-1mgDST) with variable cut-offs (above 5 µg/dL) and on elevated 24 h urinary free cortisol (UFC) levels [5–7]. To enhance sensitivity and identify milder CS, the F-1mgDST cut-off has been progressively reduced and since 2008 the diagnosis of CS has been based on the presence of at least two altered parameters among F-1mgDST ≥ 1.8 µg/dL, elevated UFC and late-night salivary cortisol (LNSC) levels [1]. Nowadays, these criteria are still considered the gold standard [8].

Recent data have suggested to increase sensitivity even in diagnosing MACS and, as a consequence, the criteria for diagnosing this asymptomatic form of hypercortisolism have shifted from a F-1mgDST cut-off of 3 µg/dL (along with at least two altered parameters among elevated UFC and/or LNSC levels) [9] to a F-1mgDST cut-off of 1.8 µg/dL without additional criteria [2], in line with the CS diagnostic threshold.

This alignment in diagnostic criteria for MACS and CS, despite their differing clinical presentations, is justified by the variable phenotypic landscape of CS. Many patients with overt CS exhibit few clinical signs, and there is a considerable overlap in symptoms between CS and MACS [10, 11]. Moreover, the frequency of comorbidities is similar in both groups, including increased risks of bone fragility, hypertension, metabolic disturbances, and elevated cardiovascular risk and mortality [12, 13].

Importantly, some studies suggest a continuum of comorbidity risk in patients with AI, with this risk increasing with the increase of F-1mDST levels [14]. Many patients with non-functioning adrenal incidentalomas (NFAI) may still face chronic consequences associated with cortisol excess. These findings raise concerns that the current F-1mgDST cut-off of 1.8 µg/dL might lack sufficient sensitivity, potentially overlooking patients with subtle yet harmful cortisol excess.

This review aims to address two key questions: (i) Is the F-1mgDST cut-off of 1.8 µg/dL still adequate for diagnosing hypercortisolism? (ii) If not, what should the appropriate F-1mgDST cut-off be?

Methods

We searched PubMed, Web of Science and Scopus between January 1990 and March 2025 using the following keywords: "adrenal incidentalomas, non-functioning adrenal incidentalomas, adrenal adenomas, non-functioning adrenal adenomas, subclinical hypercortisolism, mild hypercortisolism, less severe hypercortisolism, hidden hypercortisolism, mild autonomous cortisol secretion". The potentially eligible articles were subsequently screened.

Results

Question 1: is the F-1mgDST cut-off of 1.8 µg/dL still adequate for diagnosing hypercortisolism?

Table 1 summarizes the main findings from available studies comparing patients with NFAI to age-, body mass index (BMI)-, and gender-matched subjects without adrenal diseases, specifically focusing on potential consequences of cortisol excess. Interestingly, about 30 years ago, a small study observed higher 131-norcholesterol uptake and contralateral adrenal suppression in five patients with NFAI. This finding suggested that, despite the absence of overt hypercortisolism, the hypothalamic-pituitary-adrenal (HPA) axis was still inhibited [15]. These data align with the finding that after the removal of a NFAI some patients present a post-surgical adrenal insufficiency, which represents an indirect sign of the HPA axis inhibition and, thus, of a cortisol hypersecretion [16, 17], although a study on a Chinese population did not confirm this evidence [18]. This discordance could be related to the different inclusion criteria (i.e. patients with AI in the former studies vs. patients with subclinical hypercortisolism in the Chinese one) and to the different criteria for diagnosing hypocortisolism (i.e. stimulated cortisol levels cut-off set at 18 µg/dL, 497 nmol/L vs. 16 µg/dL, 441 nmol/L, respectively). However, in line with the possible inhibition of HPA axis activity, two studies found lower androgen excretion in patients with NFAI compared to matched controls, despite higher, though still normal, urinary free cortisol (UFC) levels [19, 20]. Similarly, Di Dalmazi and co-authors found lower circulating levels of dehydroepiandrosterone (DHEA), measured by Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS), in patients with NFAI compared to healthy controls, and suggested an independent role of reduced DHEA levels as a contributing factor to increased waist circumference [21]. This evidence is even more significant given that it is based on measurements obtained by LC-MS/MS, which is now considered the gold standard for hormone determination [22].

Table 1 Title: studies comparing the prevalence and/or incidence of the possible complications of hypercortisolism in patients with NFAI and in healthy individuals

Question A: Is F-1mgDST < 1.8 µg/dL an adequate cut-off for ruling out hypercortisolism?				
Study	Design	Reported cortisol assay	*N*° Pts/Ctrls (matching)	Main findings
Tobinaga J, 1996 (ref 15)	Cross-sectional	RIA (Baxter y Coat TM Cortisol kit)	5	Higher 131norcholesterol uptake and partial/complete contralateral adrenal suppression
Ermetici, 2008[a] (ref 41)	Prosp. Cr-sect. Ctrld.	CL (Immulite 2000)	21/18 (age, gender, BMI)	higher LVMI and diastolic dysfunction in NFAI
Yener, 2009[a] (ref 44)	Retr. Cr-sect. Ctrld.	CL (Immulite 2000)	49/18 (age, gender, BMI)	higher systolic and mean BP and higher CIMT in NFAI
Yener, 2009 (ref 40)	Retr. Cr-sect. Ctrld.	CL (Immulite 2000)	45/37 (age, gender, BMI)	higher BP, uric acid and D-dimer in NFAI
Erbil, 2009[a, b] (ref 34)	Prosp. Cr-sect. Ctrld.	SP (DPP Modular System, Roche)	35/35 (age, gender, BMI)	lower FMD of the brachial artery and increased prevalence of MS in NFAI
Peppa, 2010[a] (ref 23)	Prosp. Cr-sect. Ctrld.	N.A.	29/37 (age, gender, BMI)	higher IR, BP, dyslipidaemia, and fatty liver disease in NFAI
Eller-Vainicher, 2010 (ref 16)	Retr. Cr-sect. Interv.	IF (TDX–FLX Abbott)	60	adrenal failure after the removal of the adrenal mass in 21.5% of NFAI
Yener, 2011 (ref 35)	Prosp. Cr-sect. Ctrld.	CL (Immulite 2000)	40/22 (age, gender, BMI)	lower FMD of the brachial artery and IL-18 level in NFAI, but normal CIMT
Yener, 2012 (ref 54)	Prosp. Cr-sect. Ctrld.	CL (Immulite 2000)	38/30 (age, gender, BMI)	higher systolic and mean BP and WC in NFAI
Yener, 2012 (ref 69)	Prosp. Long.	CL (Immulite 2000)	231	increased deterioration or development of atherosclerotic risk factors in NFAI
Anderwald, 2013[a, b] (ref 51)	Retr. Cr-sect. Ctrld.	N.A.	155/25 (age, gender, BMI)	higher IR
Androulakis, 2014[a] (ref 36)	Prosp. Cr-sect. Ctrld.	RIA (DIAsource ImmunoAssays)	60/32 (age, gender, BMI)	higher IR and endothelial dysfunction in NFAI
Arduc, 2014[a, b] (ref 27)	Prosp. Cr-sect. Ctrld.	RIA (N.A.)	113/152 (age, gender, BMI)	higher WC and prevalence of HT and DM in NFAI
Vasilev, 2014 (ref 49)	Retr. Cr-sect.	N.A.	515	higher prevalence of DM, IFG and MS in NFAI than the general population
Tuna, 2014[a] (ref 28)	Prosp. Cr-sect. Ctrld.	N.A.	28/41 (age, gender, BMI)	higher prevalence of HT and CIMT in NFAI
Delibasi, 2015[a, b] (ref 45)	Prosp. Cr-sect. Ctrld.	N.A.	40/35 (age, gender, BMI)	higher IR and CIMT in NFAI
Lopez, 2016[a, b] (ref 14)	Retr. Long. Ctrld.	N.A.	166/740 (age, gender)	higher prevalent and incident DM in NFAI independent of BMI, race and smoke
Evran, 2016[a] (ref 37)	Prosp. Cr-sect. Ctrld.	CL (Beckman DXI 800 autoanalyzer)	76/33 (age, gender, BMI)	higher IR, CIMT and diastolic disfunction in NFAI
Imga, 2016 (ref 42)	Prosp. Cr-sect. Ctrld.	N.A.	51/35 (age, gender, BMI)	higher CIMT, LVMI and epicardial fat thickness in NFAI
Akkan, 2017 (ref 24)	Prosp. Cr-sect. Ctrld.	CL (Architect Abbott i2000SR)	35/35 (age, gender, BMI)	worse peripheral and central blood pressure and arterial stiffness parameters in NFAI
Arruda, 2017[b] (ref 26)	Prosp. Cr-sect. Ctrld.	CL (Immulite 2000)	40/40	higher prevalence of HT and resistant HT in NFAI
Cansu, 2017[a] (ref 48)	Prosp. Cr-sect. Ctrld.	N.A.	35/35 (age, gender, BMI)	higher cardiovascular risk factors (CIMT, PWV and AIx) in NFAI
Kizilgul, 2017[a] (ref 25)	Prosp. Cr-sect. Ctrld.	N.A.	28/40 (gender)	higher SBP, CRP, CIMT, FBG in NFAI
Krzyżewska, 2017 (ref 50)	Prosp. Cr-sect.	N.A.	131	higher prevalence of DM and IFG in NFAI than the general population
Papanastasiou, 2017[a] (ref 52)	Prosp. Long.	RIA (DIA source ImmunoAssays)	51	increased IR deterioration over time
Marina, 2018[a] (ref 53)	Prosp. Cr-sect. Ctrld.	RIA (CORT-CT2, Cisbio Bioassays)	75/30	higher IR in NFAI
Ribeiro-Cavalari, 2018[a, b] (ref 19)	Retr. Cr-sect. Ctrld.	CL (Cobas; Roche 2010)	74/90 (age, gender, BMI)	higher MS, WC and DHEAS levels in NFAI
Sokmen, 2018[a, b] (ref 43)	Prosp. Cr-sect. Ctrld.	N.A.	30/40 (age, gender, BMI)	prolonged intra- and inter-atrial conduction and increased LVMI in NFAI

Table 1 (continued)

Question A: Is F-1mgDST < 1.8 µg/dL an adequate cut-off for ruling out hypercortisolism?				
Study	Design	Reported cortisol assay	*N*° Pts/Ctrls (matching)	Main findings
Moraes, 2019[a, b] (ref 55)	Prosp. Cr-sect. Ctrld.	CL (Cobas, Roche 2010)	44/41	higher WC and MS and reduced lean mass in NFAI
Elhassan YS, 2019 (ref 66)	Metanalysis	N.A.	4121	similar mortality in NFAI and MACE pts
Emral, 2019[b] (ref 46)	Prosp. Cr-sect. Ctrld	N.A.	83/56 (age, gender, BMI)	increased IR, MS and higher CIMT in NFAI
Reimondo, 2020[a, b] (ref 30)	Prosp. Cr-sect. Ctrld.	CL (CLIA, Siemens)	20/20 (age, gender, BMI)	higher DM prevalence in NFAI
Singh, 2020 (ref 64)	Retr. Cr-sect.	N.A.	1081	higher frailty index in NFAI than that in subjects of similar age
Kim, 2020[a, b] (ref 29)	Retr. Cr-sect/Long. Ctrld.	RIA (Beckman Coulter, CA, USA)	154/462 (age, gender)	higher prevalence but not incidence of DM, HT and IR in NFAI
Akkus, 2020 (ref 38)	Cr-sect. Ctrld.	N.A.	55/49 (age, gender, BMI)	higher coronary-artery calcium score in NFAI
Li, 2021 (ref 62)	Population-based Reg.	N.A.	141/141 (age, gender)	higher prevalence of vertebral fractures in NFAI
De Paula, 2021 (ref 31)	Prosp. Cr-sect. Ctrld.	CL (Cobas, Roche 2010)	42/40 (age, gender, BMI)	more antihypertensive drugs to achieve BP control in NFAI
Athanasouli, 2021 (ref 59)	Metanalysis	N.A.	1548	higher odds of DM and higher levels of FBG and HOMA in NFAI
Araujo-Castro, 2023 (ref 20)	Prosp. Cr-sect. Ctrld.	N.A.	24/24 (age, gender, BMI)	lower excretion of androgen metabolites and higher excretion of UFC in NFAI
Dagdemir, 2023* (ref 56)	Prosp. Cr-sect. Ctrld.	N.A.	100/50 (age, BMI)	higher WC and total fat mass in NFAI
Favero, 2023 (ref 60)	Metanalysis	N.A.	4716	higher prevalence of HT, DM and MS in NFAI
Kjellbom, 2023 (ref 68)	Retr. Long. Ctrld.	IC (Cobas, Roche)	1154/3462 (age, gender)	no higher mortality in NFAI
Patrova, 2023 (ref 67)	Retr. Reg-based. Ctrld.	N.A.	17,726/124,366 (age, gender)	increased overall mortality, CV and cancer mortality in NFAI
Dogra, 2023[b] (ref 65)	Cr-sect. Ctrld.	N.A.	163/89	increased frailty in NFAI
Karatas, 2023 (ref 57)	Retr. Cr-sect. Ctrld.	CL (Unicel Dxl 800 Immunoassay System, Beckman-Coulter Inc)	134/68 (age, gender, BMI)	increased IR, MS and higher visceral adiposity in NFAI
Rebelo, 2023 (ref 32)	Prosp. Cr-sect. Ctrld.	CL (Cobas, Roche 2010)	89/64 (age, BMI)	increased prevalence of resistant HT, MS, dyslipidemia, waist-to-hip ratio in NFAI
Szychlinska, 2023 (ref 33)	Prosp. Cr-sect. Ctrld.	N.A.	48/44 (age, gender, BMI)	increased prevalence of HT and MS; higher CIMT in NFAI.
Favero, 2024 (ref 63)	Retr. Cr-sect. Ctrld.	IF (TDX-FLX, Abbott)	306/213 (age, gender, BMI)	higher prevalence of vertebral fractures in NFAI
Parasiliti-Caprino, 2024 (ref 39)	Retr. Cr-sect. Ctrld.	CL (Cobas e601, Roche)	906/1091 (propensity score)	higher ascending aorta dilation in NFAI
Alkan, 2025 (ref 61)	Prosp. Cr-sect. Ctrld.	CL (Unicel Dxl 800 Immunoassay System, Beckman-Coulter Inc)	31/44 (age, gender, BMI)	lower muscle strength, mass, quality and performance in NFAI

Table 1 (continued)

Question A: Is F-1mgDST < 1.8 µg/dL an adequate cut-off for ruling out hypercortisolism?				
Study	Design	Reported cortisol assay	*N*° Pts/Ctrls (matching)	Main findings
Boyraz, 2025 (ref 47)	Prosp. Cr-sect. Ctrld.	CL (Cobas, Roche 2010)	80/80 (age, gender, BMI)	increased ACC/AHA, FRS and CIMT in NFAI
Kim, 2025 (ref 58)	Retr. Cr-sect. Ctrld	N.A.	1354/4062 (age, gender, BMI)	higher visceral fat area and lower muscle/fat attenuation in NFAI

[a]Included in the metanalysis by Athanasouli (ref # 57)

[b]Included in the metanalysis by Favero (ref # 58)

Abbreviations: in alphabetical order

ACC/AHA Adrenal scintigraphy: performed with 131I-norcholesterol. American Heart Association/American College of Cardiology. ACS: autonomous cortisol secretion. AIx: augmentation index. CORTISOL ASSAY: method used for measuring cortisol. BMI: body mass index. BP: Blood pressure. CIMT: carotid artery intima media thickness. CRP: C-reactive protein. Ctrls: controls are subjects without adrenal adenomas. CV: cardiovascular. DHEA-S: dehydroepiandrosterone sulphate. DM: diabetes mellitus. FMD: flow- mediated dilatation. F-1 mg DST: cortisol levels after 1 mg overnight dexamethasone test. FRS: Framingham risk score. FX: fractures. HOMA: Homeostasis Model Assessment. HT: hypertension. IFG: impaired fasting glucose. IR: Insulin resistance. IL-18: Interleukin 18. LVMI: left ventricular mass index. N.A.: not available. MS: Metabolic syndrome. NFAI: non-functioning AI. PM: post-menopausal. Post-surgery adrenal insufficiency: occurrence of adrenal insufficiency after adrenalectomy in monolateral NFAI. Pts: patients. PWV: pulse wave velocity. SBP: systolic blood pressure. THB: tetrahydrocorticosterone. THE: tetrahydrocortisone. THF: tetrahydrocortisol. UFC: urinary free cortisol. WC: waist circumference. Retr: retrospective. Cr-sect: cross-sectional. Long: longitudinal. Prosp: prospective. Ctrld: controlled. Interv: interventional. Reg: register. SP: spectrophotometry. IFM: immunofluorimetry. CL: Chemiluminescence. RIA: Radioimmunoassay. IF: Immunofluorimetry. IC: Immunochemistry

Table 2 Main outcomes and cases meeting the outcomes of the studies evaluating the prevalence of the possible comorbidities of cortisol excess in NFAI

Outcome	Cases meeting the outcome	References #
Left ventricular mass increase	102	[41–43]
Arterial blood pressure	219	[23–26, 40, 54]
Arterial hypertension	494	[27–33]
Carotid intima-media thickness	518	[25, 28, 33, 37, 42, 44–48]
Flow-mediated dilation or endothelial dysfunction	1207	[24, 34–39]
Insulin resistance	857	[23, 29, 36, 37, 45, 46, 51–53, 57]
Diabetes mellitus or impaired fasting glucose levels	1099	[14, 27, 29, 30, 49, 50]
Dyslipidaemia and/or fatty liver disease	118	23, 32
BMI, Waist circumference or Waist-to-Hip ratio	458	[19, 27, 52, 54–56]
Fat mass and/or visceral adiposity	1588	[56, 57, 58]
Metabolic Syndrome	1022	[19, 33, 46, 49, 55, 57, 34]
Sarcopenia or reduced muscle function	1429	[55, 58, 61]
Bone fragility	447	[62, 63]
Frailty	1244	[64, 65]

BMI: body mass index

Since 2008, several data have tried to assess if patients with NFAI have an increased prevalence and/or incidence of hypercortisolism-related comorbidities including cardiovascular risk, glucose metabolism derangement, body composition alteration, bone fragility, frailty, and increased mortality (Table 1). Although these studies included an overall relatively small sample of cases meeting the outcomes (Table 2), if considered altogether they give an interesting picture.

As for the cardiovascular risk, some data showed increased blood pressure levels [19, 23–26] or increased prevalence of hypertension [27–33] in patients with NFAI as compared to matched controls. Interestingly, many studies demonstrated in NFAI patients an increased prevalence of altered markers of cardiovascular risk, such as D-dimer levels, flow-mediated dilation decrease or endothelial dysfunction [24, 34–40], left ventricular mass increase [41–43] and of the prevalence of the intima-media thickness increase [25, 28, 33, 37, 42, 44–48]. As far as this latter parameter, two studies from the same research group were not able to find statistically significant differences between NFAI patients and matched controls [35, 44]. However, this discordance is likely due to the small sample size of both studies ($n = 62$ and $n = 68$, respectively), as in both of them, in fact, the differences bordered on statistical significance (i.e. $p < 0.09$) and in one study, IMT was significantly elevated in AI group when compared with non-matched controls [44].

Some data also suggest glucometabolic derangements in patients with NFAI, particularly in terms of the prevalence of diabetes and/or fasting glucose levels [14, 27, 29–33, 49, 50] and insulin resistance [23, 29, 36, 37, 45, 46, 51–53]. Other studies also found an increased prevalence of dyslipidaemia ad fatty liver disease in NFAI patients than in controls [23, 32]. Aligning with these metabolic abnormalities, some studies found differences in terms of BMI, waist circumference or waist-to-hip ratio [19, 27, 32, 54–56], total fat mass and/or visceral adiposity [56–58], and, ultimately

in the prevalence of metabolic syndrome between NFAI patients and matched controls [19, 33, 34, 46, 55, 57]. Overall, based on two recent metanalyses, patients with NFAI seem to have about 1.5-fold, 1.9-fold and 2.9-fold increased odds to present insulin-resistance, arterial hypertension and metabolic syndrome, respectively [59, 60]. These two studies agreed in estimating a 2-fold increased prevalence of a glucose metabolism derangement (i.e. type 2 diabetes mellitus and/or impaired fasting glucose and/or glucose intolerance). However, the presence of an increased prevalence specifically of type 2 diabetes in NFAI is still not clear, possibly due to the low number of cases meeting the outcome ($n = 111$) available so far. Similarly, the low number of reported cardiovascular events (5 studies, 75 cases meeting the outcome) also prevents us to reliably evaluate the possible increased prevalence of cardiovascular events in NFAI patients [60].

Although even less numerous, also studies on muscle and bone health, suggest the possible presence of a relative sarcopenia and of an impaired bone health in NFAI subjects. Indeed, a reduction of muscle mass, strength, quality and performance [55, 58, 61] as well as an increased prevalence of vertebral fractures [62, 63] has been described in NFAI patients than in matched controls, in line with the finding of increased frailty in these patients as compared to what expected in matched individuals [64, 65].

Finally, data on mortality have been reported in three recent studies, giving not conclusive data. Indeed, in their metanalysis focused on patients with MACS, Elhassan and co-authors reported a similar mortality between MACS and NFAI patients [66], suggesting a possible deleterious effect of an increased cortisol secretion on survival in NFAI as reported in MACS. In line with these findings, a retrospective register-based controlled study on 17.726 AI patients and 124.366 age- and gender- matched controls reported a 1.4-fold increased risk of overall mortality, 1.2-fold increased risk of cardiovascular mortality and 1.5-fold increased risk of cancer mortality in NFAI patients, with the risk being higher in individuals below 65 years of age [67]. On the contrary, a smaller retrospective longitudinal cohort-based controlled study including 1154 patients with NFAI and 3462 age- and gender-matched controls failed to find a difference in the mortality risk in NFAI patients as compared to controls [68]. A possible explanation of this discordance could be related to the different size and design of the two studies, the former being a case-control registry study including 142.092 individuals [67], while the latter a retrospective age- and gender-controlled study on 4616 individuals [68]. Moreover, it could be possible that the relatively short median follow-up (about 6.2 years) have rendered these data less reproducible.

Some information could also be derived from longitudinal studies, even though data are limited. In a prospective study on 231 patients with NFAI, Yener and co-authors found an increased deterioration or development of atherosclerotic risk factors in NFAI than expected in the general population [54, 69]. In keeping with these findings, a subsequent prospective study on 51 NFAI patients showed an increased deterioration of insulin sensitivity over time [52] and retrospective data from 166 patients and 740 age- and gender matched controls suggested a higher incidence of type 2 diabetes in NFAI independent of age, BMI, gender, race and smoking habit [14]. At variance, in a study on 154 NFAI patients and 462 age- and gender-matched controls, Kim and co-authors failed to find and increased incidence of type 2 diabetes, insulin resistance and cardiovascular events in NFAI subjects after adjusting for age, sex, BMI and smoking and alcohol drinking status [29]. On the other hand, even patients with NFAI had increased visceral fat area and lower muscle and fat attenuation compared with controls, indicating potential cardiometabolic risks as suggested by the authors themselves [58].

Overall, the available literature suggests that patients with NFAI exhibit a higher prevalence of comorbidities potentially associated with hypercortisolism, and that they may be at increased risk of glucose metabolism deterioration over time. The insufficient data on cardiovascular events and the absence of interventional studies remain a significant limitation, weakening the strength of the evidence derived from cross-sectional and longitudinal data. Nevertheless, current findings indicate that a subset of patients with AI currently defined as NFAI may indeed have hypercortisolism. Consequently, the notion of lowering the F-1mgDST threshold for defining hypercortisolism is becoming increasingly relevant.

Question 2: what should the appropriate F-1mgDST cut-off be?

A summary of the available studies assessing the ideal cut-off of F-1mgDST for defining patients with hypercortisolism is reported on Table 3. Seven studies evaluated the threshold of F-1mgDST with the best diagnostic accuracy for identifying NFAI patients with possible comorbidities of hypercortisolism [16, 26, 36, 47, 63, 70, 71], while only two studies assessed the cut-off of F-1mgDST with the best accuracy for predicting the incidence of cortisol-related comorbidities [14, 72]. Overall, almost all studies showed that the higher the F-1mgDST levels, the higher the hypertension, insulin-resistance, type 2 diabetes, cardiovascular and fragility fracture risk, suggesting that in NFAI patients the cortisol secretion is a continuum from clearly normal to inappropriately increased cortisol levels [73, 74].

The F-1mgDST thresholds associated with the presence of the main possible cortisol-related comorbidities in

Table 3 Studies assessing the association of F-1mgDST thresholds with the prevalence and/or incidence of the possible complications of hypercortisolism in patients with NFAI or in healthy individuals

Question B: Which could an adequate F1mgDST cut-off for ruling out hypercortisolism be?				
Study	Design	Reported cortisol assay	*N*° Pts/Ctrls (matching)	Main findings
Arruda, 2017[b] (ref 26)	Prosp. Cr-sect. Ctrld.	CL (Immulite 2000)	40/40	F1mgDST of 1.03 associated with HT (cut-off 1.03 µg/dL, SN 83%, SP 83%) in NFAI
Araujo-Castro, 2023 (ref 70)	Retr. Cr-sect.	N.A.	305	F1mgDST predicts CV risk (cut-off 0.9 µg/dL) and ACS (cut-off 1.4 µg/dL) in NFAI
Favero, 2023 (ref 71)	Retr. Cr-sect.	IF (TDX-FLX Abbott; Elecsys Cortisol Immunoassay, Cobas e602, Roche)	615	F1mgDST ≥ 1.2 µg/dL associated with higher prevalence of HT and DM in nFAI
Favero, 2024 (ref 63)	Retr. Long.	IF (TDX-FLX Abbott)	85	F1mgDST > 1.2 µg/dL associated with higher incidence of fragility fractures in NFAI
Androulakis, 2014 (ref 36)	Prosp. Cr-sect. Ctrld	RIA (DIAsource ImmunoAssays)	60/32 (age, gender, BMI)	LDDST > 1.39 and 1.11 µg/dL have best predictive value for CVR and IR, respectively, in NFAI
Morelli, 2014 (ref 72)	Retr. Long.	N.A.	206	F1mgDST > 1.5 µg/dL has the best accuracy for predicting CVE (SN 77.3%, SP 50%), in NFAI
Lopez, 2016 (ref 14)	Retr. Long. Ctrld.	N.A.	166/740 (age, gender)	DM incidence higher with higher F1mgDST (trend for cut-off 1.4 µg/dL) in NFAI
Eller-Vainicher C, 2020 (ref 76)	Retr. Cr-sect. Interv.	IF (TDX-FLX Abbott)	60	F1mgDST < 1.2 µg/dL rules out hypocortisolism after the removal of the adrenal mass
Boyraz, 2025 (ref 47)	Prosp. Cr-sect. Ctrld.	CL (Cobas, Roche 2010)	80/80 (age, gender, BMI)	F1mgDST > 1.12 has the best accuracy for predicting CV risk (SN 90%, SP 78.6%) in NFAI

Abbreviations: in alphabetical order

ACS: autonomous cortisol secretion. CORTISOL ASSAY: method used for measuring cortisol. BMI: body mass index. Ctrls: controls are subjects without adrenal adenomas. CV: cardiovascular. CVE: cardiovascular events. CVR: cardiovascular risk. DM: diabetes mellitus. F-1 mg DST: cortisol levels after 1 mg overnight dexamethasone test. HT: arterial hypertension. IR: insulin resistance. NFAI: non-functioning AI. LDDST: Low-Dose Dexamethasone Suppression Test. Pts: patients. SP: specificity. SN: sensitivity. Retr: retrospective. Cr-sect: cross-sectional. Long: longitudinal. Prosp: prospective. Ctrld: controlled. Interv: interventional. N.A.: data not available. CL: Chemiluminescence. RIA: Radioimmunoassay. IF: Immunofluorimetry. IC: Immunochemistry

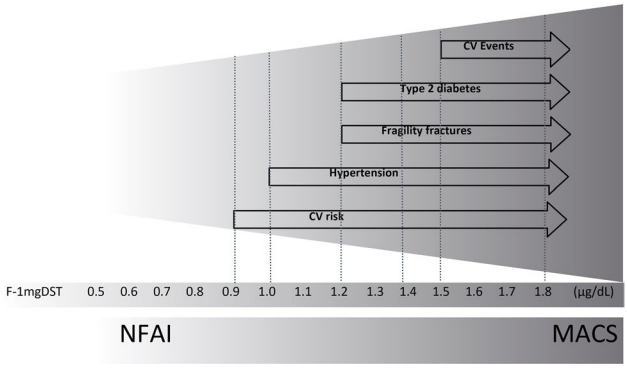

Fig. 1 The F-1mgDST thresholds associated with the presence of the main possible cortisol-related comorbidities in patients with NFAI. NFAI: non-functioning adrenal incidentalomas. MACS: mild autonomous cortisol secretion. F-1mgDST: cortisol levels after 1 mg overnight dexamethasone test

patients with NFAI is depicted in Fig. 1. In these patients, the best cut-off associated with hypertension (655 cases meeting the outcome) and type 2 diabetes mellitus (781 cases meeting the outcome) has been reported to vary between 1.03 and 1.2 µg/dL [26, 71], and between 1.2 and 1.4 µg/dL, respectively [14, 71] although the statistical significance was not reached in the study by Lopez and co-authors [14]. The presence of an increased cardiovascular risk profile (445 cases meeting the outcome) has been suggested to be associated with a F-1mgDST cut-off ranging between 0.9 and 1.4 µg/dL [36, 47, 70], while an increased risk of CVE has been shown to be associated with F-1mgDST levels above 1.5 µg/dL in a study on 206 patients with AI [72].

Considering together the studies focusing on the presence of insulin resistance (991 cases meeting the outcome), the incidence of vertebral fractures (447 cases meeting the outcome) and the occurrence of post-surgical adrenal insufficiency (60 cases meeting the outcome) as proxy for the presence of hypercortisolism in NFAI patients [36, 63, 75], the threshold for F-1mgDST levels ranged between 1.1 and 1.2 µg/dL.

Finally, it is interesting to note that the development of MACS over time in 305 patients with NFAI has been reported to be more frequent in the presence of F-1mgDST levels above 1.4 µg/dL [70]. Overall, the limited data available seem to suggest that the F-1mgDST threshold could be lowered to at least less than 1.2 µg/dL, in order to increase sensitivity and to reduce the number of patients with AI at

risk of the possible cortisol-related comorbidities and/or the development of MACS.

Conclusions

This review aimed to address two key questions: (i) Is the F-1mgDST cut-off of 1.8 µg/dL still adequate for diagnosing hypercortisolism? (ii) If not, what should the appropriate F-1mgDST cut-off be? Regarding the first question, in our opinion, several data suggest that the current threshold of 1.8 µg/dL for the F-1mgDST fails to identify all AI patients with MACS. Therefore, efforts to lower this threshold are warranted. As far as the second question, the current literature is still too scarce to identify the best cut-off of F-1mgDST for diagnosing MACS, even because the occurrence of the different cortisol-related comorbidities could be associated with a different degree of cortisol hypersecretion (Fig. 1). However, a F-1mgDST threshold of 1.2 µg/dL could be proposed.

However, it must be underlined the importance of the laboratory method used for measuring cortisol. Indeed, while several studies have highlighted the poor reproducibility of immunoassays measuring basal or stimulated cortisol [76, 77], to date, only one study focused on immunoassay performance post dexamethasone suppression, demonstrating that one in three analysed immunoassays required lowering the cut-off from 1.8 µg/dL (50 nmol/L) to 1.13 µg/dL (31.2 nmol/L) [78]. This represents an important limitation in interpreting the present data. Indeed, based on the assays used for measuring cortisol (Table 1), the evidence suggesting that NFAI patients exhibit altered hormonal and metabolic profiles, as well as an increased risk of cardiometabolic outcomes later in life, may be influenced due to immunoassay-related misclassification, potentially leading to MACS false negatives. In addition, no studies have measured cortisol by mass spectrometry-based assays, which are recognized as much more specific, sensitive and accurate than automated immunoassays from routine labs [22]. In general, it should be observed that, nowadays, a plethora of immunoassays is used in clinical laboratories, each potentially differing in performance, and for most of which no F-1mgDST cut-off has been established. Thus, there is a general need to harmonize cortisol assays, as well as dexamethasone and other steroid measurements, because without such harmonization, proposing generalized cut-off values is not feasible, and reliable sensitivity and specificity for diagnosing MACS cannot be determined. On the other hand, the fact that measuring cortisol by different immunoassays and applying the current F-1mgDST cut-off (i.e., 1.8 µg/dL or 50 nmol/L) may cause us to miss MACS in some patients, makes the reliability of this cut-off even more questionable. Indeed, a study evaluating the diagnostic performance of three immunoassay platforms (Roche Elecsys II, Abbott Alinity, and Siemens Centaur) compared to LC-MS/MS showed method-dependent variation in serum cortisol analysis during the F-1mgDST. Roche and Siemens assays aligned more closely with LC-MS/MS, while Abbott potentially caused a reduction in the sensitivity of the 1 mg DST [78]. Similarly, a recent study showed that, using the F-1mgDST cut-off of 1.8 µg/dL (50 nmol/L), both the immunochemiluminescent assays Roche Elecsys I (Roche Diagnostics) and Access Cortisol (Beckman Coulter) yielded approximately 6.5-7% possible false negatives when compared with LC-MS/MS. Additionally, Roche Elecsys I resulted in about 4% possible false positives. The optimal cut-offs were found to be 1.5 µg/dL (41 nmol/L) for Roche Elecsys I and 1.2 µg/dL (33 nmol/L) for Access Cortisol [79].

Thus, since all these methods have been used in many studies included in the present review, we cannot exclude the possibility that the diagnostic accuracy in identifying NFAI may have been influenced. Indeed, it is possible that some patients diagnosed with NFAI based on F-1mgDST levels measured by less sensitive assays might have been diagnosed with MACS if more sensitive assays had been used.

The available literature is affected by further significant limitations, including the relatively small number of cases meeting the outcome, the predominance of cross-sectional data, and the lack of intervention studies. These limitations prevent us from drawing clear-cut conclusions. On the other hand, lowering the F-1mgDST cut-off to below 1.2 µg/dL may increase the test sensitivity, allowing earlier detection of hypercortisolism. Identifying these patients through a more sensitive test could enable timely management, preventing also the possible progression to more severe disease and improving patient outcomes while reducing long-term complications. Therefore, despite the significant limitations of the current literature, it may be beneficial to monitor AI patients with F-1mgDST levels above 1.2 µg/dL over time for the potential development of hypercortisolism-related comorbidities and the possible emergence of MACS.

However, lowering F-1mgDST cut-off has disadvantages. Indeed, although a lower cut-off increases the sensitivity of the F-1mgDST, it is important to note that this could also result in more false positives. Lowering the cut-off too much could lead to diagnosing more people as having hypercortisolism, who do not actually have the condition. Therefore, any changes to the F-1mgDST cut-off should be based on clinical evidence and validated in prospective studies to ensure that the benefit of improved sensitivity outweighs the potential harm from false positives. In order to increase sensitivity without significantly reducing specificity, the

measurement of dexamethasone levels in blood [22], as well as the use of reliable methods for assessing markers of autonomous cortisol secretion, such as adrenocorticotropic hormone and dehydroepiandrosterone sulphate levels, or of new biomarkers, such as urinary steroid profile, should be widely accessible [80, 81]. As far as cortisol secretion is concerned, it is worth noting that, in recent years, a highly specific and widely available monoclonal immunoassay, calibrated against liquid chromatography-tandem mass spectrometry, has been introduced [76]. This development has led to a significant lowering of the cortisol thresholds used to diagnose adrenal insufficiency [77]. Conversely, in the context of hypercortisolism, the appropriateness of the current cut-off values for F-1mgDST, when measured using this new assay, has not yet been validated.

Finally, in patients with possible MACS, a short-term trial of cortisol-directed drugs could be hypothesized to evaluate the impact of a mild hypercortisolism on certain comorbidities, such as hypertension and type 2 diabetes. Indeed, in a patient with AI and suspected MACS, the improvement in blood pressure and/or glucose metabolism control following treatment with a cortisol-directed drug could be considered an *ex adiuvantibus* indicator of cortisol hypersecretion. In summary, lowering the cut-off for the dexamethasone suppression test below 1.2 µg/dL could help with earlier detection, better differentiation of cases, and more accurate identification of mild cortisol excess, but it requires careful consideration to avoid compromising specificity and causing unnecessary follow-up tests.

Beside the setting of patients with AI, even in some individuals with cortisol levels within the accepted normal range, evidence suggests that cortisol secretion may still be linked to typical effects of hypercortisolism. Indeed, the degree of cortisol secretion, even within the normal range, have been suggested to be associated with increased bone loss and vertebral fractures [82–84], increased incidence of cardiovascular disease [85–87]. Interestingly, metabolic health can worsen, especially in individuals with cortisol levels within the accepted normal values and genetic variations that increase glucocorticoid sensitivity, leading to poorer glucose and lipid profiles [88–94]. Finally, in addition to cortisol levels, its circadian rhythm is also crucial. Disruption of this rhythm (e.g., in night shift workers or frequent travellers) has been linked to increased metabolic and cardiovascular risks [95–97]. If these findings are confirmed, 'functional hypercortisolism' should no longer be regarded as a normal physiological condition [98].

Further studies in this field have the potential to significantly reshape the clinical management of patients with NFAI, as currently outlined in the latest European guidelines (e.g., the 2023 ESE/ENSAT guidelines) [2]. Firstly, future research could support a reclassification of NFAI, moving away from the term "non-functioning" in favour of a more nuanced, spectrum-based classification, such as "adrenal incidentaloma with low-grade autonomous cortisol secretion". Patients previously labelled as NFAI may be re-categorized as having MACS or functional adenomas based on updated diagnostic thresholds. In this regard, prospective studies could validate a lower F-1mgDST cut-off (e.g., ~ 1.2 µg/dL, 33 nmol/L) using standardized cortisol assays, and define risk-stratified thresholds that associate specific F-1mgDST levels with clinically meaningful outcomes (e.g., hypertension, type 2 diabetes, cardiovascular events). These data could ultimately lead to revise diagnostic criteria that allow for earlier identification and management of at-risk patients. Secondly, there is a clear need for personalization of follow-up strategies. Currently, patients with NFAI and no overt hormonal excess are often discharged without further monitoring. However, demonstrating that even "low-normal" cortisol levels are linked to increased cardiometabolic risk could justify stratified follow-up protocols. For example, patients with F-1mgDST values between 1.2 and 1.8 µg/dL (33–50 nmol/L) might benefit from closer monitoring and more frequent metabolic screening. This would mark a shift from the current binary classification (functioning vs. non-functioning) to a risk-based follow-up model. Thirdly, interventional trials using cortisol-lowering drugs could provide proof-of-concept that treatment improves metabolic outcomes even in patients with so-called "non-functioning" adrenal adenomas. This could support the use of an "*ex adiuvantibus*" approach, where treatment is guided by clinical response, and eventually justify therapeutic trials in selected patients based on cortisol levels and comorbidity profiles. Fourth, future studies could explore the integration of biomarkers and genomics into routine clinical assessment. Identifying genetic polymorphisms or biomarkers (e.g., urinary steroid profiles, ACTH suppression, androgen secretion) predictive of MACS progression or comorbidity development would enable personalized risk stratification and management. Fifth, standardization of laboratory assays remains a critical unmet need. The wide variability in cortisol assay performance and the lack of harmonization across laboratories contribute to inconsistent diagnoses. Large-scale studies could reinforce the need for the widespread adoption of mass spectrometry or standardized monoclonal immunoassays, the establishment of assay-specific F-1mgDST cut-offs, and the inclusion of dexamethasone measurement to verify adequate suppression and test validity. Finally, further research may also impact the surgical management of adrenal incidentalomas. At present, adrenalectomy is generally not recommended in patients with NFAI and benign imaging features. However, if future studies demonstrate that cortisol levels < 1.8 µg/dL (50 nmol/L) can still be clinically harmful, surgical criteria may need to

be broadened to include patients with biochemical evidence of cortisol excess and relevant comorbidities.

In conclusion, patients with NFAI, particularly those with F-1mgDST values above 1.2 µg/dL, may be at increased risk for hypercortisolism-related complications. In the authors opinion, while awaiting more robust evidence from large longitudinal and interventional studies, closer monitoring of these patients appears warranted to avoid missing individuals who may develop clinically significant cortisol-related comorbidities.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest. IC received consulting fees from Corcept Therapeutics and HRA Pharma.

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